510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY AND INSTRUMENT

I Background Information:

A 510(k) Number

K242388

B Applicant

Bonraybio Co., LTD.

C Proprietary and Established Names

LensHooke X12 PRO Semen Analysis System

D Regulatory Information

Product Code(s)	Classification	Regulation Section	Panel
POV	Class II	21 CFR 864.5220 - Automated Differential Cell Counter	HE - Hematology

II Submission/Device Overview:

A Purpose for Submission:

Clearance of a new device

B Measurand:

Sperm concentration (M/mL), total motility (%), progressive motility (%), morphology (%), and DNA Fragmentation Index

C Type of Test:

Analysis of semen parameters

III Intended Use/Indications for Use:

A Intended Use(s):
See Indications for Use below.

B Indication(s) for Use:
The LensHooke X12 PRO Semen Analysis System used with LensHooke Semen Test Slides is an optical device for human semen analysis which provides direct and calculated measurements for:

Sperm concentration (M/mL)
Total motility (PR+NP, %)
Progressive Motility (PR, %)
Sperm Morphology (normal forms, %)

The LensHooke X12 PRO Semen Analysis System used with LensHooke R10 Plus Sperm DNA Fragmentation Rapid Test Kit (SCD Assay) and LensHooke R11 Plus Sperm Double Strand DNA Fragmentation Rapid Test Kit (SDFR Assay) is an optical device for human semen analysis which provides direct measurement for:

Sperm DNA Fragmentation Index (DFI, %)

The LensHooke X12 PRO Semen Analysis System does not provide a comprehensive evaluation of a male's fertility status. It is an in-vitro diagnostic system intended for human semen analysis of individuals in clinical laboratories to evaluate male fertility.

C Special Conditions for Use Statement(s):

Rx - For Prescription Use Only

D Special Instrument Requirements:

LensHooke X12 PRO Semen Analysis System

IV Device/System Characteristics:

A Device Description:

LensHooke X12 PRO Semen System consists of the following components:

- LensHooke X12 PRO Semen Analysis System
- LensHooke CS3 10 μm Semen Test Slides
- LensHooke R10 Sperm DNA fragmentation test kit (SCD assay)
- LensHooke R11 Sperm double strand DNA fragmentation test kit (SDFR assay)
- LensHooke X QC Beads (For Semen) (cleared in K202089)
- LensHooke X QC Slide (For Semen)
- Self-Test Slide

- Power Cable

For CS3, R10, and R11 slides, there are two wells with 25 fields per well (5x5). Samples can be evaluated per well or across two wells. The fields evaluated are determined prior to analysis and can follow two automatic modes or customized modes, as shown in the following:

- Auto Mode 1 (CS3): six default center fields in well 1 are counted;
- Auto Mode 2 (CS3): six default center fields in well 2 are counted;
- Auto Mode 1 (DFI): all 25 fields in well 1 are counted;
- Auto Mode 2 (DFI): all 25 fields in well 2 are counted;
- Customized (either CS3 or DFI): end-users can pre-select any number or arrangement of fields they prefer. The end user may customize the mode to utilize both wells, if the concentration is expected to be low;
- For CS3, additional fields are also added by the software automatically if the count does not reach 200.

B Principle of Operation:

LensHooke X12 PRO Semen Analysis System integrates optical design and image analysis, and is combined with artificial intelligence image processing method, to fully automate analysis of semen quality including sperm concentration, motility, morphology and DNA fragmentation index. The images are captured and recorded by cameras and with image processing methods, the locations of sperms are detected. The sperm concentration is analyzed by the sperm unit density; the sperm motility is calculated by tracing sperm trajectories and the sperm morphology is calculated by comparing head and tail percentage.

LensHooke R10 Plus Sperm DNA fragmentation test kit (SCD assay) is an assay using Sperm Chromatin Dispersion (SCD) method to evaluate DNA fragmentation in human spermatozoa and analyze sperm DNA fragmentation index. The SCD assay uses unfixed semen sample (fresh, diluted, or neat samples) embedded in a melted agarose microgel which is attached to a pretreated glass slide. After DNA denaturation, lysis of nuclear proteins and staining procedures, sperms with fragmented DNA do not form the characteristic DNA halo as seen in sperms with intact DNA.

LensHooke R11 Plus Sperm double strand DNA fragmentation test kit (SDFR assay) is an assay using Sperm DNA Fragmentation Release (SDFR) method to evaluate double-stranded DNA fragmentation in human spermatozoa and assess sperm DNA fragmentation index. The SDFR Assay uses unfixed semen samples (fresh, diluted, or neat samples) embedded in a hydrogel which is attached to a pretreated slide. After lysis of nuclear proteins and staining procedures, sperms with fragmented DNA form the dispersion DNA halos which are not seen in sperms with intact DNA.

Through staining by the DNA fragmentation test kit, the DNA fragmented and non-DNA fragmented sperms are categorized. The DNA fragmentation index is calculated as the ratio described in the formula below.

$$\text{DNA fragmentation index} = \frac{\text{Number of sperms with fragmented DNA}}{\text{Number of sperms evaluated}} \times 100\%$$

C Instrument Description Information:

1. Instrument Name:

   LensHooke X12 PRO Semen Analysis System

2. Specimen Identification:

   A patient ID number can be manually entered or barcode scanned on LensHooke X12 PRO Semen Analysis System.

3. Specimen Sampling and Handling:

   The sample should be allowed to liquefy at room temperature for at least 30 minutes before testing. The test can be performed up to 1 hour after sample collection. It is recommended that users allow 2 to 7 days without ejaculation before collecting a semen sample. Condoms and lubricants should not be used when collecting a semen sample. Hands should be washed with soap and water before and after handling the semen sample.

4. Calibration:

   The LensHooke X12 PRO Semen Analysis System is factory calibrated. User calibration is not required.

5. Quality Control:

   External QC materials are provided for users of the LensHooke X12 PRO upon request by contacting customer service. Quality controls are provided as three different sperm concentration levels (negative, low positive (~25 $\times 10^6$/mL), high positive (~50 $\times 10^6$/mL)), which can be supplied in two formats: LensHooke X QC Beads (For Semen) or LensHooke X QC Slides. The QCs assess the accuracy and precision of sperm counting by providing a known target value and +/- range.

   For DNA Fragmentation Index assays (R10 Plus, R11 Plus), the positive and negative control samples are prepared as per the recommended procedures in the R10 Plus Sperm DNA Fragmentation Rapid Test Kit (SCD Assay) user manual and R11 Plus Sperm Double Strand DNA Fragmentation Rapid Test Kit (SDFR0 Assay) user manual.

V Substantial Equivalence Information:

A Predicate Device Name(s):
LensHooke X1 PRO Semen Quality Analyzer, LensHooke X1 PRO SE Semen Quality Analyzer

B Predicate 510(k) Number(s):
K202089

C Comparison with Predicate(s):

Device & Predicate Device(s):	K242388	K202089
Device Trade Name	LensHooke X12 PRO Semen Analysis System	LensHooke X1 PRO Semen Quality Analyzer
General Device Characteristic Similarities		
Intended Use/Indications For Use	The LensHooke X12 PRO Semen Analysis System used with LensHooke Semen Test Slides is an optical device for human semen analysis which provides direct and calculated measurements for: Sperm concentration (M/mL) Total motility (PR+NP, %) Progressive Motility (PR, %) Sperm Morphology (normal forms, %) The LensHooke X12 PRO Semen Analysis System used with LensHooke R10 Plus Sperm DNA Fragmentation Rapid Test Kit (SCD Assay) and LensHooke R11 Plus Sperm Double Strand DNA Fragmentation Rapid Test Kit (SDFR Assay) is an optical device for human semen analysis which provides direct measurement for: Sperm DNA Fragmentation Index (DFI, %) The LensHooke X12 PRO Semen Analysis System does not provide a comprehensive evaluation	The LensHooke X1 PRO Semen Quality Analyzer used with LensHooke Semen Test Cassette is an optical device for human semen analysis which provides direct and calculated quantitative measurements for: (1) Sperm concentration (10^6 per ml) (2) Total motility (PR+NP, %) • Progressive motility (%) • Non-Progressive motility (%) (3) Sperm morphology (normal forms, %) (4) pH value The LensHooke X1 PRO Semen Quality Analyzer does not provide a comprehensive evaluation of a male's fertility status. It is an in-vitro diagnostic system intended for human semen analysis of individuals in healthcare professional setting to evaluate male fertility.

	of a male's fertility status. It is an in-vitro diagnostic system intended for human semen analysis of individuals in clinical laboratories to evaluate male fertility.	
Technology	Desk-top unit consists of light sources, built-in video microscopy and an internal computer containing algorithms for the assessment of semen parameters.	Same
Intended User	Clinical laboratory professional	POC and Clinical laboratory professional
Parameters	Sperm concentration (M/mL), total motility (%), sperm morphology (%)	Same
General Device Characteristic Differences		
Transmission interface	HDMI/USB/Ethernet	HDMI/USB
Standard/Guidance Document	WHO 5th and 6th Edition	WHO 5th Edition
Compatible/Consumable	Semen Test Slide (CS3)	Semen Test Cassette (CS0, CS1)
Test Kits	SCD Assay (R10), SDFR Assay (R11)	N/A
Different parameters	DNA Fragmentation Index (DFI)	pH
Control Material	X QC Beads, X QC Slide	X QC Beads, X QC Reticle
Sample prep	Liquefaction/ dilution	Liquefaction/ sample cup

VI Standards/Guidance Documents Referenced:

CLSI EP05-A3 (Reaffirmed: September 2019): Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline - Third Edition

CLSI EP06-Ed2 (2020): Evaluation of the Linearity of Quantitative Measurement Procedures - Second Edition

CLSI EP07 (2018): Interference Testing in Clinical Chemistry - Third Edition

CLSI EP25-A2 (2023): Evaluation of Stability of In Vitro Diagnostic Reagents - Second edition

ISO 14971 Third Edition 2019-12 Medical devices - Application of risk management to medical devices

IEC 60601-1 Edition 3.2 2020-08 CONSOLIDATED VERSION Medical electrical equipment - Part 1: General requirements for basic safety and essential performance

IEC 60601-1-2 Edition 4.1 2020-09 CONSOLIDATED VERSION Medical electrical equipment - Part 1-2: General requirements for basic safety and essential performance - Collateral Standard: Electromagnetic disturbances - Requirements and tests

IEC 61326-1 Edition 3.0 2020-10 Electrical equipment for measurement, control and laboratory use - EMC requirements - Part 1: General requirements

IEC 61326-2-6 Edition 3.0 2020-10 Electrical equipment for measurement, control and laboratory use - EMC requirements - Part 2-6: Particular requirements - In vitro diagnostic (IVD) medical equipment

IEC 61000-3-2:2018/AMD1:2020 Amendment 1 - Electromagnetic compatibility (EMC) - Part 3-2: Limits - Limits for harmonic current emissions (equipment input current ≤16 A per phase)

IEC 61000-3-3:2013+AMD1:2017 Electromagnetic compatibility (EMC) - Part 3-3: Limits - Limitation of voltage changes, voltage fluctuations and flicker in public low-voltage supply systems, for equipment with rated current ≤16 A per phase and not subject to conditional connection

VII Performance Characteristics (if/when applicable):

A Analytical Performance:

1. Precision/Reproducibility:

Repeatability

A precision study was conducted evaluating sperm concentration, motility, progressive motility, and morphology using seven native semen samples. A separate repeatability study was conducted to evaluate the precision of the DFI on the LensHooke device. Semen samples were prepared at seven DFI values spread across the measuring range as verified by the manual method (reference). For both studies, the analysis was carried out over the course of one day by three operators using two analyzer/three test slide lot combinations. Due to the limited stability of semen samples, each “day” in the statistical analysis represents different times of day (e.g., every one hour = one “day”). Measurements were separated into five separate 1-hour time periods or “days”. The study includes three replicates per run, and two runs for every one hour, five times/day (three operators × three replicates (with three lots) × two runs (with two devices) × five times/day = 90 data points per sperm concentration/sperm motility/sperm morphology level). The sample test on well 1 at RUN 1 and test on well 2 at RUN 2. Data analyses were performed to provide repeatability, between-run, within-day, between-day, and within-laboratory. All results met the predefined acceptance criteria.

Concentration (10^6/mL)			Repeatability		Between-Run		Between-Day		Within-Laboratory	
Sample	**N**	**Mean**	**SD**	**CV%**	**SD**	**CV%**	**SD**	**CV%**	**SD**	**CV%**
1	90	6.97	0.6	8.2%	0.0	0.0%	0.1	1.0%	0.6	8.3%
2	90	75.50	6.0	7.9%	0.0	0.0%	1.1	1.5%	6.1	8.0%
3	90	231.09	8.5	3.7%	4.7	2.0%	0.0	0.0%	9.7	4.2%
4	90	74.27	4.0	5.3%	1.3	1.8%	0.0	0.0%	4.2	5.6%
5	90	65.29	3.7	5.7%	0.0	0.0%	0.0	0.0%	3.7	5.7%
6	90	93.32	7.5	8.1%	0.0	0.0%	3.2	3.4%	8.2	8.8%
7	90	63.62	4.7	7.4%	2.0	3.2%	0.0	0.0%	5.1	8.0%

Motility (%)			Repeatability		Between-Run		Between-Day		Within-Laboratory	
Sample	**N**	**Mean**	**SD**	**CV%**	**SD**	**CV%**	**SD**	**CV%**	**SD**	**CV%**
1	90	69.3	6.3	9.10%	0.4	0.60%	0.0	0.00%	6.3	9.10%
2	90	4.8	0.4	9.00%	0.1	1.90%	0.0	0.00%	0.4	9.20%
3	90	81.4	3.7	4.50%	0.0	0.00%	0.9	1.10%	3.8	4.60%
4	90	42.8	2.6	6.00%	0.0	0.00%	0.0	0.00%	2.6	6.00%
5	90	65.8	4.0	6.10%	0.7	1.10%	0.9	1.40%	4.2	6.30%
6	90	54.4	2.5	4.70%	0.6	1.00%	0.0	0.00%	2.6	4.80%
7	90	80.2	4.5	5.60%	1.9	2.30%	0.0	0.00%	4.9	6.10%

Progressive Motility (%)			Repeatability		Between-Run		Between-Day		Within-Laboratory	
Sample	**N**	**Mean**	**SD**	**CV%**	**SD**	**CV%**	**SD**	**CV%**	**SD**	**CV%**
1	90	59.2	5.7	9.60%	1.6	2.70%	0.0	0.00%	5.9	10.00%
2	90	4.1	0.4	9.70%	0.0	0.00%	0.1	2.00%	0.4	9.90%
3	90	70.0	4.1	5.90%	1.1	1.60%	0.0	0.00%	4.3	6.10%
4	90	36.8	2.6	6.90%	0.0	0.00%	0.3	0.90%	2.6	7.00%
5	90	56.9	3.9	6.90%	1.0	1.80%	0.6	1.10%	4.1	7.20%
6	90	46.9	3.1	6.60%	0.0	0.00%	0.0	0.00%	3.1	6.60%
7	90	69.0	4.7	6.80%	2.0	2.90%	0.0	0.00%	5.1	7.40%

Morphology (%)			Repeatability		Between-Run		Between-Day		Within-Laboratory	
Sample	**N**	**Mean**	**SD**	**CV%**	**SD**	**CV%**	**SD**	**CV%**	**SD**	**CV%**
1	90	7.3	0.7	9.60%	0.0	0.00%	0.0	0.70%	0.7	9.60%
2	90	2.9	0.3	9.60%	0.0	0.00%	0.0	0.00%	0.3	9.60%
3	90	8.4	0.5	5.70%	0.1	1.00%	0.1	1.10%	0.5	5.90%
4	90	4.9	0.3	6.60%	0.0	0.00%	0.0	0.60%	0.3	6.60%
5	90	12.1	0.9	7.40%	0.1	0.80%	0.0	0.00%	0.9	7.40%
6	90	7.5	0.5	6.80%	0.0	0.00%	0.1	1.50%	0.5	6.90%
7	90	15.0	1.1	7.30%	0.3	1.90%	0.3	1.90%	1.2	7.80%

DFI (R10 Plus) (%)			Repeatability		Between-Run		Within-Day		Between-Day		Within-Laboratory	
Sample	N	Mean	SD	CV %	SD	CV%	SD	CV%	SD	CV %	SD	CV%
1	90	7.92	0.6	7.90%	0.0	0.00%	0.6	7.90%	0.0	0.00%	0.6	7.90%
2	90	17.38	1.1	6.10%	0.0	0.00%	1.1	6.10%	0.0	0.00%	1.1	6.10%
3	90	24.35	0.7	3.00%	0.2	0.90%	0.8	3.10%	0.0	0.00%	0.8	3.10%
4	90	37.08	0.6	1.70%	0.0	0.00%	0.6	1.70%	0.1	0.40%	0.6	1.70%
5	90	46.90	0.7	1.50%	0.3	0.70%	0.8	1.70%	0.1	0.20%	0.8	1.70%
6	90	53.75	0.8	1.50%	0.1	0.20%	0.8	1.50%	0.2	0.40%	0.9	1.60%
7	90	67.8	0.7	1.10%	0.1	0.20%	0.7	1.10%	0.1	0.20%	0.7	1.10%

DFI (R11 Plus) (%)			Repeatability		Between-Run		Within-Day		Between-Day		Within-Laboratory	
Sample	N	Mean	SD	CV %	SD	CV%	SD	CV%	SD	CV %	SD	CV%
1	90	5.74	0.4	7.60%	0.1	2.10%	0.5	7.90%	0.0	0.00%	0.5	7.90%
2	90	16.47	1.0	6.20%	0.0	0.00%	1.0	6.20%	0.0	0.00%	1.0	6.20%
3	90	22.06	0.6	2.50%	0.2	1.10%	0.6	2.70%	0.0	0.00%	0.6	2.70%
4	90	36.38	0.7	2.00%	0.2	0.50%	0.8	2.10%	0.0	0.00%	0.8	2.10%
5	90	45.28	0.7	1.50%	0.0	0.00%	0.7	1.50%	0.0	0.00%	0.7	1.50%
6	90	55.75	1.4	2.60%	0.6	1.10%	1.6	2.80%	0.0	0.00%	1.6	2.80%
7	90	67.40	1.1	1.60%	0.0	0.00%	1.1	1.60%	0.1	0.20%	1.1	1.70%

Reproducibility

To evaluate the reproducibility performance of the LensHooke X12 PRO Semen Analysis System, a precision study was conducted using a total of three test slide lots and two analyzers at three clinical laboratory sites in the U.S. For sperm concentration, three levels of LensHooke X QC Beads control solution were evaluated. For the DNA fragmentation index (DFI) evaluation, three fresh native semen samples of various levels were utilized. Due to the limited stability of semen samples, for the DFI study each "day" in the statistical analysis represents different times of day (e.g. every one hour = one "day"). The studies included three operators × six replicates (two devices and three slides lots) × five days. For each of the three site, three lots of test slides and 2 devices were used. Each "replicate" is one well per slide. The sample test was performed on well 1 at RUN 1 and test on well 2 at RUN 2. Data analyses were performed for each site and overall to provide repeatability, between-day, between-laboratory, between-lot, and total reproducibility. All results met the predefined acceptance criteria.

Parameter [units]	N	Mean	Repeatability		Between-Day		Between Laboratory		Between Lot		Reproducibility	
			SD	CV%	SD	CV%	SD	CV%	SD	CV%	SD	CV%
Concentration (10^6/mL)	90	0.00	0.00	N/A	0.00	N/A	0.00	N/A	0.00	N/A	0.00	N/A
	90	26.41	0.91	3.4%	0.05	0.2%	0.00	0.00%	0.07	0.3%	0.91	3.4%
	90	51.00	0.63	1.2%	0.04	0.1%	0.00	0.00%	0.00	0.0%	0.65	1.3%
R10 Plus DFI (%)	90	15.92	1.08	6.80%	0.19	1.20%	0.36	2.30%	0.00	0.00%	1.17	7.40%
	90	30.43	2.13	7.00%	0.73	2.40%	0.86	2.80%	0.00	0.00%	2.30	7.60%
	90	68.45	1.30	1.90%	0.14	0.20%	0.72	1.00%	0.00	0.00%	1.56	2.30%
R11 Plus DFI (%)	90	13.41	1.19	8.90%	0.00	0.00%	0.00	0.00%	0.00	0.00%	1.28	9.50%
	90	37.21	2.51	6.80%	0.81	2.20%	1.76	4.70%	0.00	0.00%	3.13	8.40%
	90	80.66	6.73	8.30%	0.00	0.00%	0.50	0.60%	0.00	0.00%	6.82	8.50%

Between-Instrument and Between-Operator Precision

Two concurrent studies were performed to evaluate the variability between instruments and the variability between operators independently. For the between-operator study, three operators were evaluated. For the between-instrument study, three instruments were evaluated. Both evaluations included three replicates for every 1 hour, five times/day, totaling 45 data points per level. Analysis was performed to calculate the overall repeatability %CV, between-instrument %CV, and between-operator %CV, which meet the pre-defined acceptance criteria.

Concentration (10^6/mL)		Operator Precision					Instrument Precision				
		Mean	Between-Operator		Repeatability		Mean	Between-instrument		Repeatability	
Sample	N		SD	CV%	SD	CV%		SD	CV%	SD	CV%
1	45	15.68	0.0	0.0%	0.1	0.5%	15.78	0.0	0.1%	0.1	0.5%
2	45	89.39	0.8	0.8%	1.5	1.7%	52.68	0.0	0.0%	0.3	0.5%
3	45	242.14	0.3	0.1%	1.6	0.7%	237.01	0.4	0.2%	3.2	1.3%
4	45	84.03	0.0	0.0%	1.1	1.3%	75.65	0.1	0.1%	0.3	0.3%
5	45	92.11	0.4	0.4%	1.1	1.1%	65.66	0.0	0.0%	0.3	0.5%
6	45	90.18	0.0	0.0%	0.6	0.7%	80.43	0.0	0.0%	0.5	0.6%
7	45	94.97	0.0	0.0%	1.3	1.4%	57.07	0.0	0.0%	0.2	0.3%

Total Motility		Operator Precision					Instrument Precision				
		Mean	Between-Operator		Repeatability		Mean	Between-instrument		Repeatability	
Sample	N		SD	CV%	SD	CV%		SD	CV%	SD	CV%
1	45	66.1	0.0	0.0%	0.9	1.4%	60.4	0.0	0.0%	1.2	2.0%
2	45	3.9	0.0	0.0%	0.3	8.3%	4.9	0.0	0.0%	0.3	6.0%
3	45	82.4	0.2	0.3%	1.2	1.5%	80.9	0.1	0.1%	1.4	1.7%
4	45	47.3	0.0	0.0%	1.3	2.7%	45.7	0.6	1.3%	1.1	2.4%
5	45	72.5	0.4	0.5%	1.2	1.6%	76.9	0.0	0.0%	1.5	2.0%
6	45	79.2	0.0	0.0%	1.2	1.5%	73.0	0.4	0.5%	1.6	2.1%
7	45	80.7	0.0	0.0%	1.5	1.8%	79.0	0.0	0.0%	1.3	1.6%

Progressive Motility		Operator Precision					Instrument Precision				
		Mean	Between-Operator		Repeatability		Mean	Between-instrument		Repeatability	
Sample	N		SD	CV%	SD	CV%		SD	CV%	SD	CV%
1	45	52.0	0.0	0.0%	0.8	1.6%	25.0	0.0	0.0%	0.8	3.2%
2	45	0.0	0.0	N/A	0.0	N/A	1.0	0.0	0.0%	0.0	0.0%
3	45	47.9	0.1	0.2%	1.1	2.3%	53.2	0.0	0.0%	1.4	2.7%
4	45	26.1	0.0	0.0%	0.8	3.3%	21.6	0.0	0.0%	1.1	5.0%
5	45	48.4	0.2	0.3%	1.2	2.4%	38.2	0.4	1.1%	1.6	4.1%
6	45	50.4	0.0	0.0%	1.8	3.5%	34.6	0.0	0.0%	1.1	3.2%
7	45	58.6	0.0	0.0%	1.2	2.0%	47.7	0.0	0.0%	1.7	3.6%

Morphology		Operator Precision					Instrument Precision				
		Mean	Between-Operator		Repeatability		Mean	Between-instrument		Repeatability	
Sample	N		SD	CV%	SD	CV%		SD	CV%	SD	CV%
1	45	3.0	0.0	0.0%	0.0	0.0%	3.0	0.0	0.0%	0.0	0.0%
2	45	2.0	0.0	0.0%	0.0	0.0%	2.0	0.0	0.0%	0.0	0.0%
3	45	14.0	0.0	0.0%	0.2	1.5%	12.0	0.0	0.0%	0.2	1.8%
4	45	8.1	0.0	0.2%	0.3	3.1%	6.0	0.0	0.0%	0.0	0.0%
5	45	5.0	0.0	0.0%	0.0	0.0%	10.0	0.0	0.0%	0.2	2.1%
6	45	7.0	0.0	0.0%	0.2	3.0%	7.0	0.0	0.0%	0.0	0.0%
7	45	11.0	0.0	0.0%	0.1	1.4%	9.0	0.0	0.0%	0.0	0.0%

DFI (R10 Plus)		Operator Precision					Instrument Precision				
		Mean	Between-Operator		Repeatability		Mean	Between-instrument		Repeatability	
Sample	N		SD	CV%	SD	CV%		SD	CV%	SD	CV%
1	45	18.07	0.0	0.0%	0.5	3.0%	16.15	0.0	0.0%	0.3	2.1%
2	45	37.27	0.0	0.0%	0.7	2.0%	30.20	0.0	0.0%	0.6	2.0%
3	45	61.27	0.4	0.6%	1.6	2.7%	54.78	0.0	0.0%	0.7	1.4%

DFI (R11 Plus)		Operator Precision					Instrument Precision				
		Mean	Between-Operator		Repeatability		Mean	Between-instrument		Repeatability	
Sample	N		SD	CV%	SD	CV%		SD	CV%	SD	CV%
1	45	5.22	0.1	1.2%	0.2	4.2%	4.40	0.0	0.0%	0.2	5.2%
2	45	37.22	0.1	0.1%	0.4	1.0%	24.51	0.0	0.0%	0.3	1.0%
3	45	45.30	0.0	0.1%	0.3	0.7%	59.36	0.0	0.0%	0.5	0.8%

2. Linearity:

Linearity for sperm concentration was evaluated using one analyzer and three slide lots. Semen samples were prepared at 11 sperm concentrations ranging from 0–400×10^6/mL, verified using reference methods LensHooke X1 and manual microscope. The mean and SD of results were calculated. Regression analysis was used to verify the linear range. Sperm concentration was demonstrated to be linear from 0.2–360 ×10^6/mL.

3. Analytical Specificity/Interference:

The potential interference of various substances on LensHooke X12 concentration results were evaluated by using three sperm concentration levels (0–20 M/mL, 50–100 M/mL, and 100–200 M/mL). The following 11 interfering substances were tested in the study: vitamin B, testosterone, yeast, E. Coli, RBC, WBC, urine, saliva, agglutination of semen sample, D-norgestrel, and β-estradiol. Samples were tested in five replicates on one analyzer using three lots of test slides. Results of the test group were compared to the control group. Study results showed that all tested interfering substances met the acceptance criteria, and no significant interference was caused by the tested substances.

A separate interference study was conducted to evaluate the interference on the DFI of the LensHooke X12 device. The potential interference of various substances on LensHooke X12 DFI results were evaluated by using two sperm DFI levels (R10 Plus:<20% and ≥20%; R11

Plus: <14% and ≥14%). The following 20 interfering substances were tested in the study: WBC, pH 6.4, pH 7.8, pH 9.2, vitamin B12, testosterone, yeast, E. coli, RBC, urine, saliva, agglutination, D-norgestrel, B-estradiol, clomiphene citrate, follicle-stimulating hormone (FSH), coenzyme Q10, conjugated bilirubin, unconjugated bilirubin and yellow pigment (Sunset Yellow FCF, Merck Cat# 465224). Samples were tested in five replicates on one analyzer using three lots of test reagents. Results of the test group were compared to the control group. Study results showed that all tested interfering substances met the acceptance criteria, and no significant interference was caused by the tested substances.

4. Assay Reportable Range:

Sperm concentration: 0.4–300 M/mL
Total Motility: 0–100%
Progressive Motility: 0–100%
Morphology: 0–100%
Sperm DNA fragmentation index (DFI): 0–100%.

5. Traceability, Stability, Expected Values (Controls, Calibrators, or Methods):

Sample Stability

Stability of semen samples was determined with three samples at different concentration levels. The semen samples were stored at room temperature. At time zero (0 hour), the samples were tested with nine replicates to establish the baseline. These samples were tested again (n=9) at various timepoints and for each time point, the results were compared to the baseline results, and the percent difference was calculated to meet the predefined acceptance criteria. The results support a 1-hour semen sample stability duration for traditional semen parameters (CS3) analysis and an 8-hour semen sample stability duration for DFI analysis.

Reagent Stability

A stability study was conducted to determine the shelf-life of the LensHooke Semen Test Slide in real-time (at 2°C and 30°C). Three semen samples with varying sperm concentrations were tested in three replicates using one analyzer and three lots of test slides. All results were compared against the reference method LensHooke X1 PRO Analyzer. Results support an 18-month shelf-life claim at refrigerated and room temperature conditions (2°C and 30°C).

6. Detection Limit:

A study was conducted to determine the detection limits of the LensHooke X12 PRO Semen Analysis System. Semen samples were taken from volunteers and two interval ranges were prepared: sperm-free seminal plasma and low concentration seminal plasma. Semen was centrifuged to obtain sperm-free seminal plasma to a concentration of ~0 M/mL (blank sample) as verified by manual microscope. Low concentration seminal plasma was prepared by diluting semen to a concentration of ~0.4 M/mL as verified by manual microscope. Blank and low-level samples were divided into five aliquots and tested in three replicates (low-level sample) or four replicates (blank sample) once a day for three days, using two lots of test

cassettes and one analyzer. Results were calculated, and the detection limits were determined to be:

Limit of Blank (LoB) = 0 M/mL
Limit of Detection (LoD) = 0.07 M/mL
Limit of Quantitation (LoQ) = 0.37 M/mL.

7. Assay Cut-Off:

See Reference Range study, section VII.E.

8. Accuracy (Instrument):

See method comparison, section VII.B.1.

9. Carry-Over:

Not applicable.

B Comparison Studies:

1. Method Comparison with Predicate Device:

A method comparison study was conducted to evaluate the performance of the LensHooke X12 PRO Semen Analysis System when used by intended users following the instructions in the package insert. The study was performed at three US sites, with a total of ten operators for semen analysis and sperm DNA fragmentation analysis. Following collection of the specimen, the user analyzed the sample on the LensHooke X12 PRO Semen Analysis System. After recording the results, a different trained technologist at the study site performed semen analysis on the sample using either the predicate (i.e., the LensHooke X1 PRO Semen Quality Analyzer for concentration, motility and morphology) or the reference method (i.e., manual microscope for DNA fragmentation analysis as recommended in the WHO 6th edition). A total of 130 semen specimens spanning the analytical measuring range were analyzed by using the Passing-Bablok regression. All results met the predefined acceptance criteria.

Parameter [Units]	N	Result Range	Slope (95% CI)	Intercept (95% CI)	R^2
Concentration [10^6/mL]	130	0.0–264.1	0.96 (0.93, 1.00)	-0.22 (-1.16, 0.67)	0.98
Total Motility [%]	130	0.0–92.0	1.00 (0.98, 1.04)	-1.00 (-2.27, 0.10)	0.98
Progressive Motility [%]	130	0.0–88.0	1.00 (0.96, 1.28)	0.50 (-0.15, 1.90)	0.97
Morphology [%]	130	0.0–13.0	1.00 (1.00, 1.00)	0.00 (0.00, 0.00)	0.95
R10 Plus DFI [%]	130	0.0–55.18	1.01 (0.98, 1.04)	-0.40 (-0.84, 0.10)	0.99
R11 Plus DFI [%]	130	0.0–55.36	1.00 (0.96, 1.04)	-0.04 (-0.46, 0.51)	0.97

2. Matrix Comparison:

The matrix comparison study was conducted to assess the comparison of fresh neat semen samples and diluted semen samples on the LensHooke X12 PRO. For the DFI evaluations, a

total of three semen samples were included in the study where paired neat semen samples were diluted with 0.01M PBS (control) or a sperm extender HTF (Human Tubal Fluid Medium) at 5x, 10x, 15x, 30x, and 60x dilution. For the traditional parameters' evaluation, a total of three semen samples were included in the study where paired neat semen samples were diluted with seminal plasma (control), or a sperm extender (SpermRinse, LifeGlobal HTF Total) at 2x and 4x dilution. Samples included low, near cutoff, and normal concentration levels. The results were compared to the control results, and the percent bias was calculated and met the predefined acceptance criteria. The study results support the use of 0.01M PBS or sperm extenders (HTF) as diluents during analysis on LensHooke X12 PRO (CS3, R10 Plus, R11 Plus), as described above.

C Expected Values/Reference Range:

A reference interval study was performed to determine the reference intervals for sperm DNA fragmentation index using the R10 Plus and R11 Plus kits on the LensHooke X12 PRO device. Semen samples from 120 healthy male adult subjects were evaluated at five clinical sites for the study (1 OUS, 4 US). The reference intervals were established with the non-parametric bootstrap method following CLSI EP28-A3c, by computing the lower reference limit (2.5th percentile) and the upper reference limit (97.5th percentile). The 95% reference interval for the SCD (R10 Plus) method was 4.16 to 35.07 %, and for the SDFR (R11 Plus) method was 1.90 to 28.57 %.

Based on the 90% confidence intervals (CI) of the upper reference limits from the study data, the low reference DNA Fragmentation Index (DFI) cut-off is 32.10% (R10 Plus) and 24.07% (R11 Plus).

For traditional semen parameters (CS3), the lower limit of the reference range for parameters Sperm Concentration, Total Motility (%), Progressive Motility (%), and Morphology (%) were directly reported as the 5th percentile in the *WHO Laboratory Manual for the Examination and Processing of Human Semen,* 6th Edition (Table 8.3).

D Other Supportive Instrument Performance Characteristics Data:

1. Capture Mode Evaluation:

   An evaluation was conducted to assess the comparison of different capture modes on the LensHooke X12 PRO, to demonstrate the equivalence of Auto-modes to the Custom Mode. Semen samples were included in the study at various levels including above, below, and near the cut-off, including four samples for DFI, six samples for concentration, five samples for motility, and three samples for morphology evaluations. Results from Auto-mode were compared to eight Custom Mode designs, intended to represent the worst-case scenarios. The percent bias was calculated by comparing the results obtained from the Auto-modes to different Custom Modes. The results demonstrate that custom mode configurations produce equivalent results compared to auto-mode, validating use of custom mode on LensHooke X12 PRO (CS3, R10 Plus, and R11 Plus).

VIII Proposed Labeling:

The labeling supports the finding of substantial equivalence for this device.

IX Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.